VIII. SUMMARY

This thesis deals with the relationship between structure and function of hemocyanin of the Roman snail, *Helix pomatia*. In Chapter II the properties of arthropodan and molluscan hemocyanins have been summarized. The differences between the hemocyanins of the two phyla support the suggestion that they originated independently.

Hemocyanins are respiratory proteins, which bind one oxygen molecule per two copper atoms. When oxygenated, hemocyanins are blue, when deoxygenated, they are colorless. The properties of oxy- and deoxyhemocyanin can be explained by a model in which the valence of copper is not defined.

The methods used in the experiments are described in Chapter III.

The association-dissociation behavior of α-hemocyanin has been studied by ultracentrifugal analysis (Chapter IV). Above pH 7 and below pH 5 whole molecules (102 S) dissociate into half (64 S) and tenth (20 S) molecules. At alkaline pH values further dissociation into twentieth (13 S) molecules is observed. The dissociation is a cooperative process. The twentieth molecules, with a mol.wt. of about $4.5 \cdot 10^5$ daltons, are the smallest biologically active components that can be obtained.

The dissociation is reversible in the pH range 4 – 11. Reassociation is induced by readjusting the pH to 5 – 7 or, at alkaline pH values, by the addition of bivalent cations.

The association-dissociation behavior does not obey the rules valid for simple equilibria, so there must be a certain heterogeneity in the bonds between the structural units.

Whole molecules are dissociated into halves by hydrostatic pressure; this results in an elevated baseline between the two components in the schlieren pattern.

The experiments about the binding of oxygen and copper are described in Chapter V. Reintroduction of monovalent copper into apo-α-hemocyanin leads to complete return of the properties of native α-hemocyanin. The presence of the copper atoms in hemocyanin is required for the reassociation of tenth and twentieth molecules to whole and half molecules. In the reconstitution process the copper atoms are randomly distributed over the empty binding sites of apo-α-hemocyanin. All copper binding sites are thus equivalent and grouped in pairs; each pair forms a functional group. Upon storage for a few months, part of the copper atoms in every molecule is oxidized to

Cu(II). This affects the oxygenation behavior.

The oxygenation curves of fresh α- and β-hemocyanin are non-hyperbolic at alkaline pH values in the presence of calcium and magnesium ions. There are marked differences in the influences of the pH on the oxygenation curves between the two compounds.

Hemocyanin was succinylated in varying degrees with succinic anhydride (Chapter VI). Not only the amino groups, and the hydroxyl groups of tyrosine, serine and threonine were acylated, but also the hydroxyl groups of the carbohydrate moiety.

Succinylation induces dissociation of hemocyanin. Even at very low degrees of succinylation dissociation into twentieths occurred; more exhaustive succinylation did not lead to smaller components.

Sedimentation and viscosity measurements, as well as electron microscopy, showed that these succinylated components had undergone considerable unfolding.

By progressive succinylation an increasing part of the copper can be removed by EDTA; the residual copper remains fully functional. Since one molecule of oxygen is bound per two atoms of copper this means that both copper atoms of a functional group are labilized simultaneously.

The cooperativity of the functional groups which is observed in native hemocyanin is completely lost by the introduction of succinyl groups.

On the basis of these results and on electron microscopic studies a tentative model for the subunit organization of *Helix pomatia* hemocyanin is proposed (Chapter VII).